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TECHNICAL MANUSCRIPT 496

A RAPID BIOASSAY
FOR SIMULTANEOUS IDENTIFICATION
AND QUANTITATION OF PICLORAM
IN AQUEOUS SOLUTION

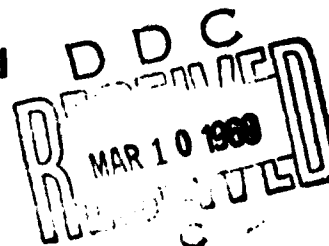
Charles P. P. Reid
Woodland Hurtt

FEBRUARY 1969

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A RAPID BIOASSAY FOR SIMULTANEOUS
IDENTIFICATION AND QUANTITATION OF
PICLORAM IN AQUEOUS SOLUTION

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ABSTRACT

A simple bioassay technique is described that offers a rapid and sensitive means for the simultaneous detection and quantitative determination of picloram (4-amino-3,5,6-trichloropicolinic acid) in micro-quantities of aqueous solution. When lettuce seed were placed on small squares of filter paper moistened with picloram solutions, the concentration of picloram was related to the degree of growth inhibition of root and hypocotyl elongation 72 hours after initiation of the test. Inhibition was a useful parameter in determining quantities of picloram ranging from 0.036 μg to 7.2 μg . Growth stimulation occurred from 0.00072 μg to 0.0072 μg . After paper chromatographing plant exudates containing C^{14} -labeled picloram, location and quantities of picloram on the chromatograms were determined by the lettuce bioassay and compared with determinations by the methods of UV light sensitivity, C^{14} 4 π strip scanning and C^{14} dilution calculations. Rf values determined by all methods were identical, and the quantitative determination by the bioassay agreed closely with calculations based on the amount of C^{14} detected by liquid scintillation counting.

I. INTRODUCTION*

The herbicide picloram (4-amino-3,5,6-trichloropicolinic acid) has been the subject of much research in the past several years because of its unique properties. Although essentially comparable to 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) in characteristics of absorption, translocation and soil-leaching, picloram is more effective on many broad-leaved plants.³ It causes distortions in growing tissue and other formative effects³ and has been observed to cause growth promotion in stem sections in much the same way as 2,4-D and other auxin-like growth regulators.¹ Investigations on modes of action of picloram at sublethal doses in plants necessitated the development of a sensitive assay for very low concentrations of active ingredient.

A simple bioassay technique has therefore been developed that offers a rapid and sensitive method for detection and quantitative determination of picloram in micro-quantities of aqueous solution. Numerous bioassay methods have been used over the years for the detection of herbicides. Frankland and Wareing² described a technique using lettuce seedlings to detect gibberellin-like substances in plant extracts. The growth inhibition of cucumber seedlings has been used for determinations of 2,4-D.⁵ A bioassay for determination of picloram in solutions and soil was described by Leasure.⁴ The technique described here offers several advantages with respect to length of time, amount of test solution, ease of identification, and determination of small absolute amounts of picloram.

II. MATERIALS AND METHODS

Lettuce seed (Lactuca sativa var. Grand Rapids) were placed on small squares of filter paper moistened with aqueous solutions containing picloram or on moistened squares cut from paper chromatograms.

To determine the range of sensitivity of the bioassay, lettuce seed were treated with various concentrations of picloram. Three-hundred microliters of picloram solution ranging from 10^{-4} M to 10^{-8} M were applied to 1-inch squares of Whatman No. 20 paper. This resulted in absolute amounts of picloram per square ranging from 7.2 μ g to 0.00072 μ g. Three uncovered 35-mm petri plates, each containing one square of paper

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with 10 lettuce seeds, were placed in a covered 100-mm petri plate. The 100-mm plates were then enclosed in plastic boxes and maintained in a controlled environment chamber at 25 C and 80 to 90% RH. A 16-hour photoperiod with approximately 350 ft-c of illumination at box-top level was provided by a mixture of fluorescent and incandescent lighting. After 72 hours, primary root and hypocotyl elongation were measured for each germinated seed. Five replications of 10 seeds each were used for each concentration.

Lettuce seed were placed on squares cut from paper chromatograms to demonstrate the practicability of the bioassay in identifying picloram in plant exudates or extracts. As a means of verification, known quantities of C^{14} -labeled picloram (labeled at the carboxyl position) were chromatographed and identified by appropriate methods in conjunction with the bioassay procedures. To obtain plant exudate containing C^{14} -labeled picloram, 10 bean plants (*Phaseolus vulgaris* var. Black Valentine) were root-treated for 3 hours with 10^{-6} M picloram in Hoagland's nutrient solution containing a predetermined quantity of C^{14} -labeled picloram. The plants were then transferred to aerated nutrient solution and detopped and xylem exudate was collected from the cut stems for 24 hours. The exudate was grouped into two collections of five plants each (exudates A and B). A 0.5-ml aliquot was removed from each collection and counted in a liquid scintillation spectrometer. The total amount of picloram in the exudate was then calculated from the ratio of C^{14} -labeled picloram in the treatment solution to the C^{14} -labeled picloram in the exudate. The two exudate collections were evaporated under vacuum to 25 μ l each and spotted on Whatman No. 20 chromatography paper. Five microliters of C^{14} -labeled picloram were also spotted and cochromatographed with the exudates. Descending chromatography was employed with acetone and water (90:10 v/v). Papers were cut into 1-inch-wide strips, viewed under 2537 Å UV light, and monitored with a Tracerlab 4 π strip scanner for detection of C^{14} spots. Strips were then cut into 1-inch-long segments and each segment was placed in a 35-mm petri plate and moistened with 300 μ l of glass-distilled water for the lettuce seed bioassay. After 72 hours, root and hypocotyl elongation measurements were made of the 10 lettuce plants on each segment.

III. RESULTS AND DISCUSSION

When values for root and shoot elongation were plotted against log concentration of picloram, the resulting curve was sigmoid and very similar to the relative cumulative frequency of a normal distribution. When the same data were plotted on log probability graph paper (Fig. 1), it was possible to fit a straight line to the points. At very low concentrations from 0.00072 μ g to 0.0072 μ g, growth stimulation occurred, while at concentrations greater than 0.0072 μ g, inhibition resulted. Inhibition was therefore a very useful parameter in determining picloram concentrations from 0.036 μ g to 7.2 μ g, the latter limit approaching complete inhibition of growth.

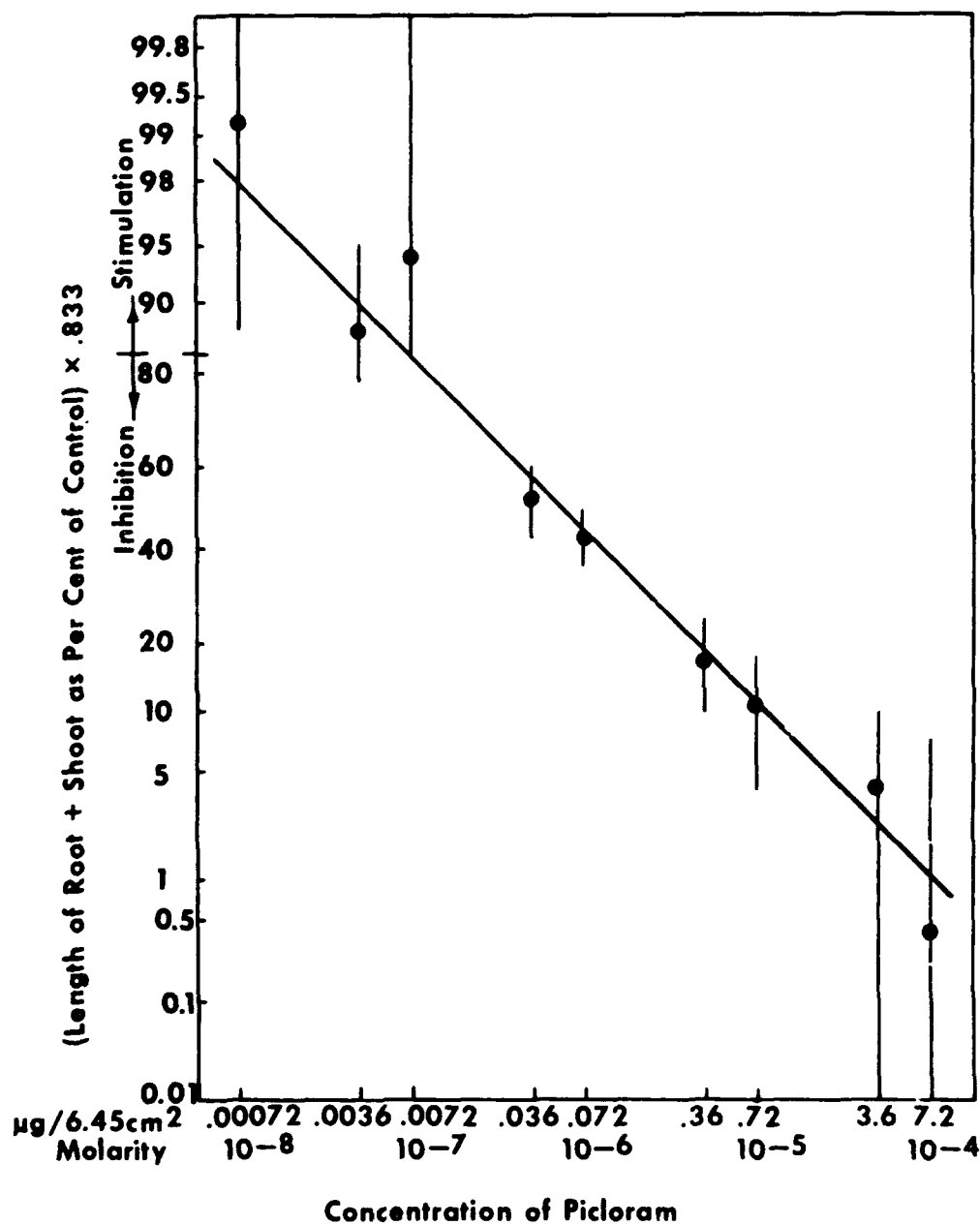


FIGURE 1. Growth of Primary Root and Shoot of Germinating Lettuce Seed Exposed for 72 Hours to Different Concentrations of Picloram. Vertical bars represent the standard error of difference between means of control and treatment based on per cent of control. Growth response is plotted on a probability scale.

The data resulting from the assay for picloram on paper chromatogram strips are presented in Figure 2. The reciprocal of the average combined length of the root and shoot of the 10 seeds on each segment was plotted in relation to its R_f value. This was also compared with R_f values of the spots determined by C^{14} detection and by the presence of spots sensitive to UV light. The locations of picloram as determined by the three methods of C^{14} count rate peaks, UV spots, and lettuce growth inhibition all agreed well with corresponding R_f values of approximately 0.62. Although the C^{14} count rate peaks of the exudates occurred at the same R_f value as the C^{14} -labeled picloram standard, the exudate spots determined under UV were much larger than the UV spot of the standard. The lettuce seed bioassay showed some inhibition over the entire length of the exudate UV spots.

Calculation of the ratios of labeled to unlabeled picloram indicated that the total amounts of picloram in the chromatogram spots of exudates A and B were 0.597 μg and 0.506 μg , respectively. The 5 μl of C^{14} -labeled picloram used as the standard contained 1.195 μg . Measurements of the lettuce placed on the chromatogram spots were compared with a standard curve of inhibition versus picloram concentration based on three replications of 10 seeds each for each concentration of 0.00072 μg to 7.2 μg . For exudate A (0.597 μg), an average elongation of 2 mm corresponded to a value between 0.36 μg and 0.72 μg as determined from the standard curve; for exudate B (0.506 μg), 3 mm elongation, a value of about 0.36 μg ; and for the picloram standard (1.195 μg), 1 mm elongation, a value between 0.72 μg and 3.6 μg . Greater accuracy in determining amounts of picloram can be obtained by diluting test solutions or reducing their volume under vacuum to arrive at concentration that would be within the more sensitive area of the standard inhibition curve.

Trials were conducted with five Whatman chromatography papers: No. 1, 3, 20, 540, and 542. Number 20 proved the most suitable in our procedures.

The primary advantages of this bioassay technique are its simplicity, rapidity (less than 72 hours), and requirement for only very minute quantities of test solution. Separation and identification of picloram by paper chromatography are accomplished simultaneously with quantitative determinations. Although 300 μl of test solution were used, it would be very feasible to use as little as 50 to 100 μl if smaller squares of filter paper were used. In addition to the above described applications of the technique, it could conceivably be used as a convenient device for detection of picloram in soil solutions, leachates, or aqueous systems such as streams and lakes.

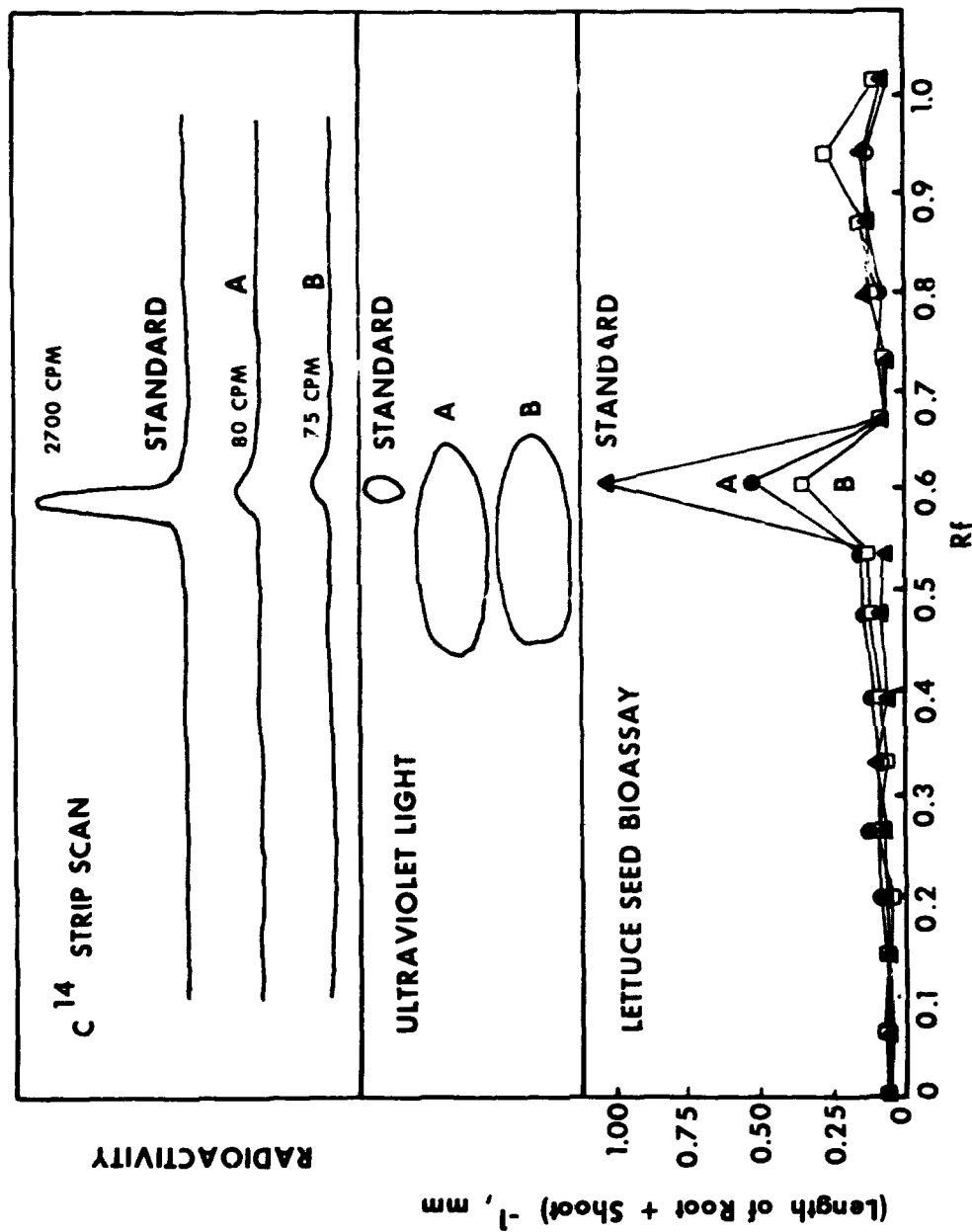


FIGURE 2. Identification of Picloram in Plant Exudates (A and B) Chromatographed on Paper with C^{14} -Labeled Picloram (Standard) by Three Methods: (i) C^{14} 4 π Strip Scanning, (ii) UV Light Sensitivity, and (iii) Lettuce Seed Bioassay.

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